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Purpose: Synovitis is evident in a substantial subpopulation of patients with early osteoarthritis (OA) and has been associated with development of pathophysiology. Recently we have shown that adipose-derived stem cells (ASC) inhibit joint destruction after local application to knee joints with experimental OA. We now explored the effect of synovitis on the immunomodulatory capacity of ASCs after local administration in two experimental OA models differing in synovitis.
Methods: ASCs were isolated from fat surrounding the popliteal lymph nodes. ASCs were injected into knee joints after induction of collagenase-induced OA (CiOA) characterized by synovitis and surgically induced destabilized medial meniscus (DMM) model in which synovitis is scant. Synovial activation, chondrogenesis in ligaments and osteophytes were measured using histology. Cytokines in synovial washouts and serum were determined using Luminex. Active TGFβ was measured using the CAGAluc assay.
Results: ASCs injected into knee joints at different time-points after induction of DMM (day 7, day 14 or day 7 and day 14) had no effect on development of ligament damage or osteophyte formation. In contrast, ASC treatment of collagenase-induced OA knee joints, rapidly inhibited synovitis and ligament damage when administered at day 7 after induction. Washouts of synovium taken at different time points after injection of ASCs (6 hrs, day 2, day 14 and day 42) showed significantly decreased levels of IL-1β and S100A8/A9 already at 48 hrs after ASC treatment. No effect was found on levels of active TGFβ. Serum levels of S100A8/A9 were significantly decreased (85% lower) at day 14 whereas IL-1 levels were not detectable anymore at that time-point. Next, we explored the effect in CiOA in a condition with less synovial inflammation. Synovial thickness at day 42 was 62% lower when compared to the former study. Injection of the same dose of ASCs at day 7 after induction of CiOA, only marginally inhibited synovial thickening when measured at day 42. Serum levels of S100A8/A9 were low at day 14 (around 50 ng/ml compared to 800 ng/ml in the first experiment) and were not altered by the ASC treatment. Chondrogenesis in collateral and cruciate ligaments but also osteophyte formation at the bone margins was also not diminished.
Conclusions: Synovial activation rapidly drives anti-inflammatory effects of ASCs after local administration in murine OA knee joints with synovitis protecting against development of ligament damage and osteophyte formation.

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IDENTIFICATION AND CHARACTERIZATION OF NOVEL STEM/PROGENITOR CELLS IN RAT ADULT ARTICULAR CARTILAGE

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Purpose: Several recent studies suggested that a progenitor cell population which resides in cartilage exhibits stem cell relevant markers, shows a phenotypic plasticity and generates chondrogenic, adipogenic, and osteogenic lineages. They also provided some evidences of a stem/progenitor cell niche within the articular cartilage. However, there is still no definitive identity of these cells understood clearly in terms of their surface markers and specific characters. The aim of this study is to identify and characterize stem/progenitor cells exist in adult articular cartilage in rats based on their differences in surface marker expression before culture expansion.
Methods: Chondrocytes were isolated from rat adult cartilage in a conventional manner using a collagenase treatment. Isolated cells were subjected to flow cytometry to investigate expression pattern of selected surface markers of mesenchymal stem cells (CD29, CD34, CD44, CD49e, CD73, CD90, CD105, CD106 and CD166). MSCs markers which showed low expression in chondrocytes were used to sort out a sub-population of cells, which was assumed to be a stem/progenitor cells in the cartilage. The sub-population of cells isolated was further examined for cell identity, multipotent differentiation ability, telomerase activity and surface marker profiles.
Results: Analysis for the MSCs markers revealed that CD49e and CD90 were not expressed highly in uncultured rat chondrocytes. Rare populations of CD49e- or CD90-positive cells were isolated successfully showed better proliferation ability than unsorted total population of cells. In particularly, they showed differentiation potential into different lineages; CD49e-positive cells had higher adipogenic potential, and CD90-positive cells had higher chondrogenic potential than each other. They also expressed most of MSCs markers on their surface but the expression level of CD49e and CD90 was decreasing along with passages after monolayer culture. Immunohistochemical analysis showed that CD49e-positive cells lined particularly along the superficial layer of rat articular cartilage.
Conclusions: These results suggest that CD49e-positive and/or CD90-positive cells could be a stem/progenitor cells in rat articular cartilage. Further studies are needed to prove their identity and physiological role in vivo.
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MSC BASED THERAPY FOR SEVERE OSTEOARTHRITIS OF THE KNEE. A PHASE 1 DOSE ESCALATION TRIAL

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a- **Purpose:** Among the degenerative diseases associated with aging, osteoarthritis (OA) is the most common pathology which affects 16% of the female population over 65 years. ASC are adult stem cells exhibiting functional properties that have open the way for cell-based clinical therapies. Primarily, their capacity of multilineage differentiation has been explored in a number of strategies for skeletal tissue regeneration. More recently, ASC have been reported to exhibit immunosuppressive as well as healing capacities, to improve angiogenesis and prevent apoptosis or fibrosis through the secretion of paracrine mediators. Up to now, no therapeutic option exists to obtain a sustainable improvement of joint function beside knee arthroplasty. This prompted us to propose adipose-derived mesenchymal stromal cells (ASC) as a possible cell therapy.
b-**Methods:** We used 2 pre-clinical models of OA, and showed that a local injection of ASC allowed a reduction of synovitis, reduction of osteophytes, joint stabilization and reduction of the osteoarthritic score. This work was completed by toxicology data showing the excellent tolerance of the local injection of ASC and biodistribution showing the persistence of cells after 6 months in immunodeficient murine model. In addition, quality control and tolerability of the injection of ASC led to the approval by AFSSAPS in France and by the PEI in Germany to conduct the phase I clinical trial. The patient received a single injection of autologous ASC, 15 days after lipoaspiration (2.106, 107 or 5.107 cells) through intra-articular injection.
c- **Results:** The ADIPOA project started in January 2010 with the goal to develop a new cell-based strategy for patients suffering from knee OA. In this open-label phase I trial, we included 18 patients with severe OA of the knee in failure of conventional therapies (62.5% were KL IV) at two sites, Montpellier and Wurzburg. Mean age was 61 years, with a 10 years history of knee OA. The primary outcome measure of effectiveness was patient-reported WOMAC pain subscores by VAS in the affected knee at week 12. Secondary outcome measures included Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International (OMERACT OARSI) responses. We observed a decrease of the VAS Pain (73±11 mm day 0 to 32±23 month 3), and of WOMAC (50±18 to 25±7 month 3).
d-**Conclusions:** This study confirms the feasibility and safety of local injection of autologous cells from adipose tissue and suggests that the most effective dose was 107 autologous cells.The ADIPOA research teams performed successfully the phase I clinical trial in France and Germany. A phase IIB controlled trial is scheduled to confirm the clinical benefit of this strategy.